

ASHMET.002A1

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : John G. Babish et al.
Appl. No. : 09/919,349
Filed : July 31, 2001
For : COMBINATIONS OF
SESQUITERPENE LACTONES
AND DITERPENE TRIPOXIDE
LACTONES FOR SYNERGISTIC
INHIBITION OF
CYCLOOXYGENASE-2
Examiner : Michelle C. Flood
Group Art Unit : 1654

DECLARATION OF JOHN G. BABISH, Ph.D. UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, John G. Babish, Ph.D. declare and state:

1. I am an inventor of the above-identified application and am familiar with the specification, claims and file history thereof.

2. I wish to provide evidence of synergy of compounds in a combination for inhibition of PGE₂ biosynthesis in target macrophage cells. In particular, the combination of parthenolide and triptolide in a certain range of ratios were shown to have a synergistic effect. Outlined below is a description of experiments to demonstrate the synergism of the compounds.

3. In the example presented below, I illustrated inhibition of PGE₂ biosynthesis in target macrophage cells for multiple combination of parthenolide and triptolide ranging from ratios of parthenolide:triptolide of 1000:1 to 1:1000. These combinations in the range of 100:1 to 1:100 provided synergistic inhibitory activity of COX-2 mediated PGE₂ biosynthesis.

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4. In the experiments described below, parthenolide and triptolide were obtained from Sigma (St. Louis, MO).

5. RAW 264.7 cells used in this example and treatment of the cells including cell plating were identical to cells and procedures previously described. Test materials were formulated to contain parthenolide:triptolide in ratios of 1000:1, 500:1, 100:1, 50:1, 1:1, 1:50, 1:100, 1:500 and 1:1000, respectively.

6. The procedure followed were that essentially described by Hamburg, M. and Samuelsson, B. (*J. Biol. Chem.* 1971. 246, 6713-6721); however, a commercial, non-radioactive procedure was employed (Cayman Chemical, Ann Arbor, MI), as described in the specification at page 36, line 11 to page 40, line 8.

7. Quantification of the combination index (CI) for the tested combinations of parthenolide and triptolide was performed using CalcuSyn (BIOSOFT, Ferguson, MO) as was described fully in the specification of the present patent application. Using the designation of CI = 1 as a merely additive effect, for mutually exclusive compounds that have the same mode of action or for mutually non-exclusive drugs that have totally independent modes of action we obtain the following relationships: CI < 1, = 1, and > 1 indicate synergism, additivity and antagonism, respectively.

8. Statistical analysis of inhibition of COX-2 biosynthesis of PGE₂ in the RAW 264.7 cell model for the nine test combinations of parthenolide and triptolide is presented in TABLE 1. The CI for IC₅₀, IC₇₅ and IC₉₀ are tabulated for each ratio of test materials. Additionally, the mean of the CI for these three locations on the dose-response curve is also presented as a parameter indicative of synergy throughout the complete dose-response curve of the ratio tested.

9. TABLE 1. Combination index values for nine ratios of parthenolide:triptolide

Test Material Ratios	Fraction Parthenolide	Fraction Triptolide	Combination Index			Mean
			IC ₅₀	IC ₇₅	IC ₉₀	
1000:1	0.999	0.001	1.93	1.89	1.91	1.91
500:1	0.998	0.002	1.86	1.56	1.60	1.67
100:1	0.990	0.010	0.005	0.402	0.520	0.309
50:1	0.980	0.020	0.033	0.573	0.700	0.435
1:1	0.500	0.500	0.180	0.642	0.138	0.320
1:50	0.020	0.980	0.191	0.061	0.539	0.263
1:100	0.010	0.990	0.353	0.265	0.652	0.423
1:500	0.002	0.998	1.07	1.18	1.49	1.25
1:1000	0.001	0.999	1.05	1.28	1.49	1.27

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10. As can be seen in TABLE 1, the ratios of test material from 100:1 to 1:100 are described as synergistic by the CI parameter. The low values for CI indicate extremely strong synergy between parthenolide and triptolide within these ratios. Unexpectedly, only one percent of either parthenolide or triptolide in the combination of the two agents produces an inhibition of PGE₂ biosynthesis greater than either material alone at the respective concentration. Also unexpectedly, this synergy exists over the entire dose-response range of the combinations as indicated by the mean CI values less than one. CI's are greater than 1.0 at the extremes of the ratios, 1000:1, 500:1, 1:500 and 1:1000, actually indicating antagonism. Thus, unexpected results are observed across the entire range of ratios for parthenolide:triptolide combinations between 100:1 and 1:100.

11. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Respectfully submitted,

Dated:

8-27-03

By:



John G. Babish, Ph.D.

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